

## Cyclin D1 splice variant and risk for non-Hodgkin lymphoma

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Received: 17 February 2006 / Accepted: 1 April 2006 / Published online: 17 June 2006  
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**Abstract** To investigate the role of cell cycle gene variations in lymphomagenesis, we evaluated associations (odds ratios [OR] and 95% confidence intervals [CI]) in polymorphisms from seven candidate genes in 1,172 non-Hodgkin lymphoma (NHL) cases and 982 population-based controls. The cyclin D1 (*CCND1*) splice variant G870A (rs603965) increased NHL risk

( $OR_{AA} = 1.4$ , 95% CI = 1.1–1.8,  $P$ -trend = 0.021), which was consistent for four B-cell subtypes. As *CCND1* expression indicates poor NHL prognosis, our results, if true, would support its potentially dual importance in NHL etiology and survival.

### Introduction

Cell cycle dysregulation is critical for carcinogenesis. A number of genes relevant for progression from the G1 to the S phase of the cell cycle have been identified and perturbation of their function is known to be relevant for hematopoietic malignancies. Chromosomal aberrations and translocations occur within key cell cycle regulatory genes in lymphomas, including cyclin D1 (*CCND1*). Cyclin D1 plays an important role in B-cell activation and differentiation, and its expression helps delineate post-germinal center activation of B-cells.

Genetic variations within cell cycle genes with transcriptional and functional relevance have been reported, but their role in NHL etiology remains largely unexplored except for a few reports (Hou et al. 2005; Zhang et al. 2005). We therefore evaluated twelve single nucleotide polymorphisms (SNPs) from seven candidate genes (*BCL6*, *CCND1*, *CCNH*, *CDKN2A*, *CHEK1*, *LMO2*, *TERT*) in our large US-based multi-center case-control study of non-Hodgkin lymphoma (NHL).

### Patients, materials and methods

The study population has previously been described in detail (Chatterjee et al. 2004). Briefly, cases included

**Electronic Supplementary Material** Supplementary material is available to authorised users in the online version of this article at <http://dx.doi.org/10.1007/s00439-006-0212-3>.

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1,321 patients with newly diagnosed NHL identified in four Surveillance, Epidemiology, and End Results registries (Iowa; Detroit, MI, USA; Los Angeles, CA, USA; Seattle WA, USA) aged 20–74 years between July 1998 and June 2000 without evidence of HIV infection. All cases were histologically confirmed and coded according to the International Classification of Diseases for Oncology, 2nd edition (Harris et al. 1994) and subsequently updated to the 3rd edition. Population controls ( $n = 1,057$ ) were identified by random digit dialing (< 65 years) and from Medicare eligibility files ( $\geq 65$  years) and matched to cases on age, sex, and race at each study site. Written informed consent was obtained from all participants, in accordance with US Department of Health and Human Services guidelines. This study was approved by the institutional review boards at the NIH and at each participating Surveillance Evaluation and End Results (SEER) site (Iowa, Seattle, Los Angeles, and Detroit).

We evaluated the 1,172 cases (89%) and 982 controls (93%) for whom biological samples were obtained for genotyping (Wang et al. 2006). DNA was extracted from blood ( $n = 773$  cases and 668 controls) using Puregene Autopure DNA extraction kits (Gentra Systems, Minneapolis, MN, USA) and from buccal cells ( $n = 399$  cases and 314 controls) by phenol-chloroform extraction (Garcia-Closas et al. 2001). As previously reported, genotype frequencies in our controls did not differ by whether blood or buccal samples were provided (Bhatti et al. 2005).

We selected seven genes (12 SNPs) with known a priori evidence of laboratory or clinical relevance to hematopoietic diseases (Table 1). Genotyping was conducted using the Taqman platform (Core Genotyping Facility, Advanced Technology Corporation, Gaithersburg, MD, USA). Sequence data and assay conditions

are provided at <http://www.snp500cancer.nci.nih.gov>. Quality control specimens included 40 replicates from two blood donors and 100 duplicates from study subjects. Agreement for QC samples was  $\geq 99\%$  for all assays. Departure from Hardy–Weinberg equilibrium as calculated by Chi square and measured as  $P < 0.05$  was assessed for each SNP in the control population stratified by race; we found that no SNPs departed from Hardy–Weinberg equilibrium in our study.

We calculated odds ratios (OR) and 95% confidence intervals (CI) for each genotype with NHL, using the homozygous wild-type genotype as the referent group. We calculated the  $P$ -trend based on the three-level ordinal variable (0, 1, 2) of homozygote wild-type, heterozygote, and homozygote variant. Finding no significant differences in risk estimates by stratified analysis (age, sex, race, study center), we present estimates adjusted for age (< 54, 55–64, 65+ years), sex, race (white, black, other), and study center, as these were all study design variables. Unconditional logistic regression (for NHL) and polytomous regression (for subtypes) were conducted using SAS 8.2 (SAS Institute, Cary, NC, USA).

## Results

The *CCND1* G870A splice variant (rs603965) was associated with increased NHL risk ( $OR_{AG} = 1.1$ , 95% CI = 0.9–1.3,  $OR_{AA} = 1.4$ , 1.1–1.8,  $P$ -trend = 0.021) (Table 2). When stratified by cell lineage, the risk remained elevated for B-cell lymphomas ( $OR_{AG} = 1.1$ , 95% CI = 0.9–1.4,  $OR_{AA} = 1.4$ , 1.1–1.9,  $P$ -trend = 0.018). Elevated risks for T cell lymphomas were not statistically significant. Among the four most common B-cell lymphoma subtypes in our study, increased risks

**Table 1** Cell cycle genes and single nucleotide polymorphisms evaluated in the NCI-SEER multicenter case-control study of non-Hodgkin lymphoma

Gene	Name	Chromosomal location	SNP rs number	Base pair change	Amino acid change
<i>BCL6</i>	B-cell CLL/lymphoma 6	3q27	rs1056932	Ex6–195C>T	N387N
<i>CCND1</i>	Cyclin D1	11q13	rs678653 rs603965	Ex5+852C>G Ex4–1G>A	3'UTR P241P
<i>CCNH</i>	Cyclin H	5q13.3-q14	rs2266690	Ex8+49T>C	V270A
<i>CDKN2A</i>	Cyclin-dependent kinase inhibitor 2A	9p21	rs3731249 rs11515 rs3088440	Ex3–16A>G Ex4+43C>G Ex4+83T>C	A148T 3'UTR 3'UTR
<i>CHEK1</i>	CHK1 checkpoint homolog	11q22-q23	rs506504	Ex13+76A>G	I471V
<i>LMO2</i>	LIM domain only 2	11p13	rs3740617 rs2038602	Ex6+106A>G Ex5+70T>C	K121K I37I
<i>TERT</i>	Telomerase reverse transcriptase	5p15.33	rs2736098 rs2853690	Ex2–659G>A Ex16+203C>T	A305A 3'UTR

**Table 2** Association of cell cycle gene polymorphisms with (a) NHL overall, B-cell, and T-cell lymphomas and (b) B-cell subtypes: diffuse large B-cell lymphoma (*DLBCL*), follicular, marginal zone and small lymphocytic lymphoma (*SLL*) (adjusted for age, race, sex, and study center)

SNP	Genotype	Control n (%)	NHL n (%)	OR (95% CI)	B-cells n (%)	OR (95% CI)	T-cells n (%)	OR (95% CI)	
<b>a</b>									
rs603965	GG	328 (35)	337 (30)	1.0 (ref)	265 (29)	1.0 (ref)	26 (37)	1.0 (ref)	
	AG	458 (49)	548 (49)	1.1 (0.9–1.3)	455 (51)	1.1 (0.9–1.4)	27 (38)	0.7 (0.4–1.3)	
	AA	142 (15)	226 (20)	1.4 (1.1–1.8)	180 (20)	1.4 (1.1–1.9)	18 (25)	1.6 (0.8–3.1)	
	AG or AA	600 (65)	774 (70)	1.2 (1.0–1.4) <i>P</i> -trend = 0.021	635 (71)	1.2 (1.0–1.5) <i>P</i> -trend = 0.018	45 (63)	0.9 (0.5–1.5) <i>P</i> -trend = 0.33	
SNP name	Genotype	DLBCL n (%)	OR (95% CI)	Follicular n (%)	OR (95% CI)	Marginal zone n (%)	OR (95% CI)	SLL n (%)	OR (95% CI)
<b>b</b>	GG	110 (32)	1.0 (ref)	81 (31)	1.0 (ref)	20 (22)	1.0 (ref)	40 (28)	1.0 (ref)
	AG	161 (47)	1.0 (0.7–1.3)	131 (49)	1.0 (0.8–1.4)	54 (59)	1.9 (1.1–3.2)	81 (56)	1.4 (0.9–2.1)
	AA	73 (21)	1.4 (1.0–2.0)	53 (20)	1.3 (0.8–1.9)	17 (19)	1.8 (0.9–3.7)	23 (16)	1.3 (0.7–2.2)
	AG or AA	234 (68)	1.1 (0.8–1.4) <i>P</i> -trend = 0.13	184 (69)	1.1 (0.8–1.5) <i>P</i> -trend = 0.29	71 (78)	1.9 (1.1–3.1) <i>P</i> -trend = 0.052	104 (72)	1.4 (0.9–2.0) <i>P</i> -trend = 0.28

were observed and significant for diffuse large B-cell lymphoma (DLBCL) ( $OR_{AA} = 1.4$ , 95% CI = 1.0–2.0) and marginal zone lymphomas ( $OR_{AG \text{ or } AA} = 1.9$ , 95% CI = 1.1–3.1) (Table 2).

There were no statistically significant differences in risk estimates when stratified by age, race or sex. No SNP or haplotype associations were found in *BCL6*, *CCNH*, *CDKN2A*, *CHEK2*, *LMO2*, or *TERT* (Supplemental Tables 1 and 2).

## Discussion

*CCND1* encodes cyclin D1, a key cell cycle regulatory protein signaling the transition from G1 to the S phase of the cell cycle. Cyclin D1 overexpression and *CCND1* translocations with IgH occur in mantle cell lymphoma, multiple myeloma, and B-cell chronic lymphocytic leukemia (Rabbits 1998). Importantly, the *CCND1* G870A polymorphism confers an alternate splice site resulting in two messenger RNA transcripts (Howe and Lynas 2001); the A allele which codes for isozyme b has previously been associated with acute lymphoblastic leukemia (Hou et al. 2005). Interestingly, the DLBCL risk increase observed with the *CCND1* G870A polymorphism may also be important as cyclin D1 expression has been reported to confer worse prognosis for DLBCL (Zhang et al. 1999). We note that the risk increase was most pronounced in marginal zone lymphomas, which, like DLBCL, is considered a more aggressive NHL subtype.

Our results suggest a potential role for *CCND1* polymorphisms in lymphomagenesis. Because we

cannot exclude the possibility of false positive results, replication of our findings and further haplotype analyses are needed. However, if our observations are verified, they would support a dual role for *CCND1* in NHL etiology and survival.

**Acknowledgements** This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, with Public Health Service (PHS) contracts N01-PC-65064, N01-PC-67008, N01-PC-67009, N01-PC-67010, and N02-PC-71105.

## References

- Bhatti P, Sigurdson AJ, Wang SS, Chen J, Rothman N, Hartge P, Bergen AW, Landi MT (2005) Genetic variation and willingness to participate in epidemiologic research: data from three studies. *Cancer Epidemiol Biomarkers Prev* 14:2449–2453
- Chatterjee N, Hartge P, Cerhan JR, Cozen W, Davis S, Ishibe N, Colt J, Goldin L, Severson RK (2004) Risk of non-Hodgkin's lymphoma and family history of lymphatic, hematologic, and other cancers. *Cancer Epidemiol Biomarkers Prev* 13:1415–1421
- Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, Bender PK, Beck JC, Le Marchand L, Lum A, Alavanja M, Hayes RB, Rutter J, Buetow K, Brantton LA, Rothman N (2001) Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiol Biomarkers Prev* 10:687–696
- Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, Delsol G, Wolf-Peeters C, Falini B, Gatter KC (1994) A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 84:1361–1392
- Hou X, Wang S, Zhou Y, Xu Z, Zou Y, Zhu X, Han M, Pang T, Han ZC (2005) Cyclin D1 gene polymorphism and suscepti-

- bility to childhood acute lymphoblastic leukemia in a Chinese population. *Int J Hematol* 82:206–209
- Howe D, Lynas C (2001) The cyclin D1 alternative transcripts [a] and [b] are expressed in normal and malignant lymphocytes and their relative levels are influenced by the polymorphism at codon 241. *Haematologica* 86:563–569
- Rabbitts TH (1998) LMO T-cell translocation oncogenes typify genes activated by chromosomal translocations that alter transcription and developmental processes. *Genes Dev* 12:2651–2657
- Wang SS, Davis S, Cerhan JR, Hartge P, Severson RK, Cozen W, Lan Q, Welch R, Chanock SJ, Rothman N (2006) Polymorphisms in oxidative stress genes and risk for non-Hodgkin lymphoma. *Carcinogenesis*
- Zhang A, Ohshima K, Sato K, Kanda M, Suzumiya J, Shimazaki K, Kawasaki C, Kikuchi M (1999) Prognostic clinicopathologic factors, including immunologic expression in diffuse large B-cell lymphomas. *Pathol Int* 49:1043–1052
- Zhang Y, Lan Q, Rothman N, Zhu Y, Zahm SH, Wang SS, Holford TR, Leaderer B, Boyle P, Zhang B, Zou K, Chanock S, Zheng T (2005) A putative exonic splicing polymorphism in the BCL6 gene and the risk of non-Hodgkin lymphoma. *J Natl Cancer Inst* 97:1616–1618